A FOSSIL RECORD FOR GROWTH REGULATION: THE ROLE OF AUXIN IN WOOD EVOLUTION[1]

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ABSTRACT

Appreciation for the role of ontogeny in plant evolution has been heightened by advances in studying development using molecular techniques, with a growing number of specific structural features now understood in terms of the genetic, regulatory, and biochemical mechanisms by which they are produced. Paleontological approaches to plant development provide a vehicle for extending that understanding to the ontogeny and evolution of whole organisms through time. Recent studies have shown that developmentally diagnostic features can be identified in the fossil record, where they represent fingerprints for gene-mediated regulatory pathways. The first paleontological evidence for the regulation of cambial activity via the polar axial flow of auxin consists of circular patterns of tracheary elements above buds and branch junctions in the wood of the 375-million-year-old fossil progymnosperm *Archaeopteris* Dawson. That evidence strongly supports homology of secondary vascular tissues in progymnosperms and seed plants, and monophylesis of the lignophytes (progymnosperms and seed plants). Similar anatomical patterns at the same position have now been identified in the wood of tree-sized fossil equisetophytes and arborescent lycophytes that belong to independent lineages, demonstrating that a similar mechanism involving the regulation of secondary vascular tissue production by the polar axial flow of auxin characterizes those clades as well. These data imply that the wood in lignophytes, lycophytes, and equisetophytes originated in conjunction with the parallel evolution of regulation of secondary tissue production by auxin in each clade. The independent origins of secondary vascular tissue in three major clades of Paleozoic plants sensu Kenrick and Crane (1997) reveal evolutionary patterns that are not represented in the living flora and illuminate promising avenues for combining future paleontological studies with molecular and genetic studies to substantially impact our understanding of the role of developmental regulation in vascular plant evolution.

Key words: Evolution, fossils, polar auxin flow, spiral vascular tissues, wood development, xylem.

The rise of classical genetics during the 1920s and 1930s fostered a fundamental advancement in Darwinian evolutionary theory, with the resulting modern synthesis placing biological evolution on a solid genetic footing (Gould, 1994). As a consequence, the focus of the study of evolution shifted from organisms to populations of organisms, and the definition of evolution was modified from changes in the structure of organisms through time to changes in relative gene frequencies through time (Gould, 1994). This modification of focus is appropriate because it is DNA (not individual organisms) that is modified through time to provide the continuity of biological evolution. Another consequence of the modern synthesis was the separation of studies of evolutionary patterns from studies of the evolutionary process. Molecular phylogenetics notwithstanding, evolutionary patterns are established primarily by changes in organisms through time (a major role of paleontology; Kenrick & Crane, 1997), whereas studies of evolutionary process focus primarily on the population genetics and the population biology of living organisms (Howell, 1998).

Recent advancements in developmental genetics and the rise of molecular developmental biology have, for the first time, allowed us to understand structural features in light of the regulatory mechanisms by which structure is patterned. For example, Reinhardt et al. (2003) have shown how auxin accumulation in the shoot apical meristem stimulates leaf outgrowth and thus controls phyllotaxy. Various works are beginning to reveal gene expression patterns that give rise to morphological variation in leaves (Chuck et al., 1996; Hareven et al., 1996; Chen et al., 1997; Kim et al., 2003; Gleissberg et al., 2005; Harrison et al., 2005).

Because we are now able to ascribe particular structural features of plants to the influence of specific genes, ontogenetic studies from the fossil record have the potential to combine with knowledge of molecular developmental pathways and allow us to infer much more precisely the evolution of tissues, organs, and whole organisms through time. Although at first glance

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paleontology seems to offer us scant potential for inferring plant development at the genetic and biochemical levels, a closer look exposes a sound theoretical and empirical foundation for the approach (Gould, 1977; Rothwell, 1987; Stein, 1993; Sanders et al., 2007) and illuminates an opportunity to reincorporate evolutionary patterns from paleontological studies into the mainstream of evolutionary process. As compared to most animals, the potential for the excellent preservation of sequences of developmental processes in fossil plants is common because the plant skeleton occurs at the cellular level (in the form of the cell wall) instead of at the organismal level (as in most animals). Consequently, virtually all plant cells are readily preserved in the paleontological record (Stewart & Rothwell, 1993; Taylor & Taylor, 1993) and available to studies of developmental biology.

The fossil record of vascular plants provides an impressive array of evidence about the ontogeny of tissues, organs, and whole organisms, as well as for the evolutionary pathways by which organs and species of organisms have evolved (Stewart & Rothwell, 1993; Bateman & DiMichele, 2002; Kenrick, 2002). Traditional ontogenetic studies of fossil plants emphasize topics such as the development of shoot systems (Delevoryas, 1964; Scheckler, 1978), growth architecture of sporophytes (Eggert, 1961; Trivett, 1993; Tomescu et al., 2006), primary and secondary meristems (Good & Taylor, 1972; Cichan, 1985), ovule ontogeny (Rothwell, 1971), and microgametophyte development (Millay & Eggert, 1974; Taylor & Rothwell, 1982). Other paleobotanical studies model apical growth (Stein, 1993), infer leaf blade development in relation to meristematic activity deduced from vein patterns (Boyce & Knoll, 2002), hypothesize regulatory mechanisms for vascular differentiation (Stein, 1993; Boyce & Knoll, 2002), and explore the evolution of meristematic activity in relation to structural changes brought about by experimental studies of gene activity in living plants (Sanders et al., 2007).

These strategies for inferring ontogeny from fossils recently have been extended to the investigation of developmental regulatory mechanisms by which the fossilized structures were produced (Rothwell & Lev-Yadun, 2005). Together with the theoretical approaches introduced above, that study illuminates the exciting potential for developmental regulatory mechanisms to be recognized and characterized from the paleontological record. In the present study, we examine and provide an example of the broad potential for fossil plants to provide developmental regulatory data and to infer the evolution of tissues, organs, and whole organisms. The secondary xylem of Paleozoic fossil lycophytes and equisetophytes has been examined to determine if specific evidence for the regulation of cambial activity by polar axial auxin flow (e.g., circular tracheary elements) extends beyond the lignophytes to other euphyllophytes and to lycophytes (Fig. 1). Circular patterns of tracheary elements are present in lignophytes, other euphyllophytes, and lycophytes but vary considerably in the extent and positions of their development. These data are evaluated in relation to the known positions of deviations of the direction of the polar auxin flow in living vascular plants (Lev-Yadun & Aloni, 1990), with the goal of interpreting the evolution of regulatory mechanisms for secondary vascular tissues across the major fossil clades of vascular land plants.

Materials and Methods

Anatomically preserved fossils of woody lycophytes and equisetophytes preserved in carbonate concretions known as coal balls (Rothwell, 2002) were sectioned in tangential view at nodes using the well-known cellulose acetate peel technique (Joy et al., 1956) to expose the patterns of tracheary elements above branches and wide leaf-trace rays. These include the Middle Pennsylvanian (ca. 310 million years old) lycophyte plant *Paralycopodites brevifolius* (Williamson) DiMichele from the Lewis Creek locality in eastern Kentucky (Phillips, 1980), the Upper Pennsylvanian (ca. 295 million years old) lycophyte plant *Chaloneria cormosa* Pigg & Rothwell from the Steubenville locality in eastern Ohio (Phillips, 1980), and a Middle Pennsylvanian (ca. 310 million years old) species of the equisetophyte morphotaxon *Arthropitys* Goeppert from the Lewis Creek locality. Peels were mounted on microscope slides under cover slips in the mounting medium Eukitt (O. Kinder, Germany; distributed by Calibrated Instruments, Inc., Hawthorne, New York) and examined using transmitted and/or reflected light. Images were captured with Microlumina (Leaf Systems, Inc., Southborough, Massachusetts) and Photophase (Phase One Industries, Copenhagen, Denmark) digital scanning cameras mounted on a copy stand or a Leitz Aristophot (Berlin, Germany) bellows camera, and processed using Adobe Photoshop CS (www.adobe.com). Specimens, peels, and microscope slides are housed in the Ohio University Paleobotanical Herbarium. Microscope slides bear acquisition numbers 18,137–13,148.

Results

PALEONTOLOGICAL EVIDENCE FOR THE DEVELOPMENTAL REGULATION OF WOOD—INITIAL WORK

Evidence for polar auxin regulation of vascular cambial activity recently has been recognized in the

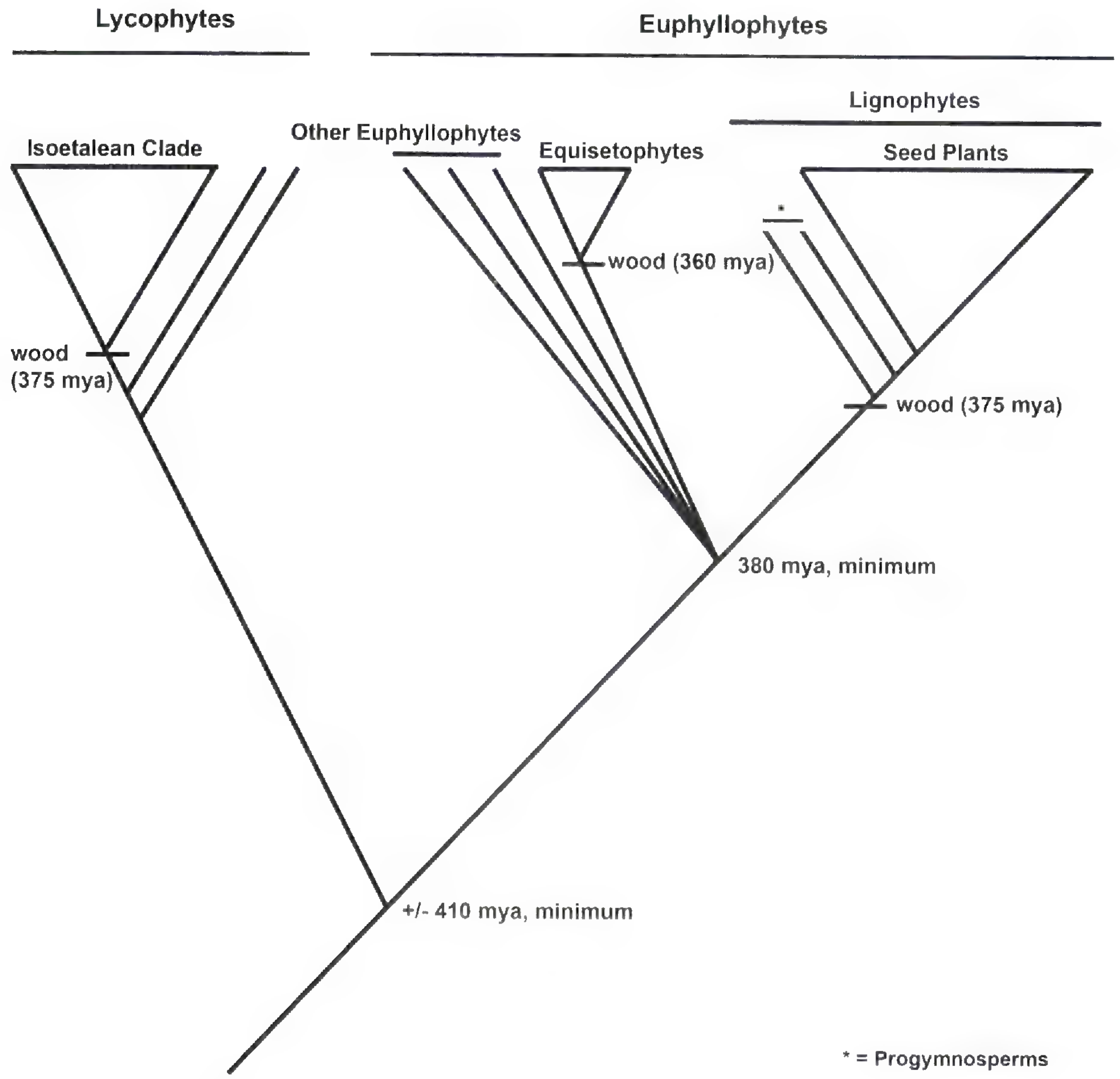

Figure 1. Simplified phylogenetic tree of vascular plants showing currently recognized relationships among lycophytes and euphyllophytes. Euphyllophyte relationships reflect strict consensus of most recent competing hypotheses (Rothwell, 1999; Pryer et al., 2001). Minimum ages for divergence of major clades are placed at relevant nodes. Hash marks on branches indicate oldest evidence for secondary growth in lycophytes, equisetophytes, and lignophytes. Note that the divergence of lycophytes from euphyllophytes occurred at least 35 million years before evidence for the origin of secondary growth in either clade and that lignophytes also appear to have diverged from equisetophytes long before the appearance of secondary vascular tissues in either. (Data primarily from Stewart & Rothwell, 1993; Taylor & Taylor, 1993; Kenrick & Crane, 1997; Rothwell, 1999; and Pryer et al., 2001.)

375-million-year-old tree trunk *Callixylon whiteanum* Arnold (Rothwell & Lev-Yadun, 2005). *Callixylon whiteanum* is a morphospecies for woody stems of the progymnosperm tree *Archaeopteris* Dawson (Trivett, 1993). Tangential sections of *C. whiteanum* wood show circular patterns of tracheids immediately above branches (Fig. 2A) that are comparable to patterns in living seed plants (Rothwell & Lev-Yadun, 2005). Because the circular patterns of living plants are produced by interruption of polar auxin flow above an obstruction in the cambium of woody dicots and conifers (Sachs & Cohen, 1982), such patterns may be regarded as a structural fingerprint for the regulation of vascular tissue development by polar axial auxin flow (Hejnowicz & Kurczyńka, 1987; Lev-Yadun & Aloni, 1990) in *Callixylon* Zallesky and other fossils as well (Fig. 2B).

ISOETALEAN (RHIZOMORPHIC) LYCOPHYTES

Giant, much-branched, tree-sized relatives of the modern quillwart *Isoetes* L. were dominant canopy trees in the equatorial wetlands of the late Paleozoic Era (360–300 million years ago [Ma]; Stewart & Rothwell, 1993). Such plants are commonly assigned to the Lepidodendrales of the isoetalean clade (Stewart & Rothwell, 1993) and are referred to as rhizomorphic because their much-branched rooting system (i.e., rhizomorph) is actually a shoot modified for rooting (Rothwell & Erwin, 1985). In a pioneering

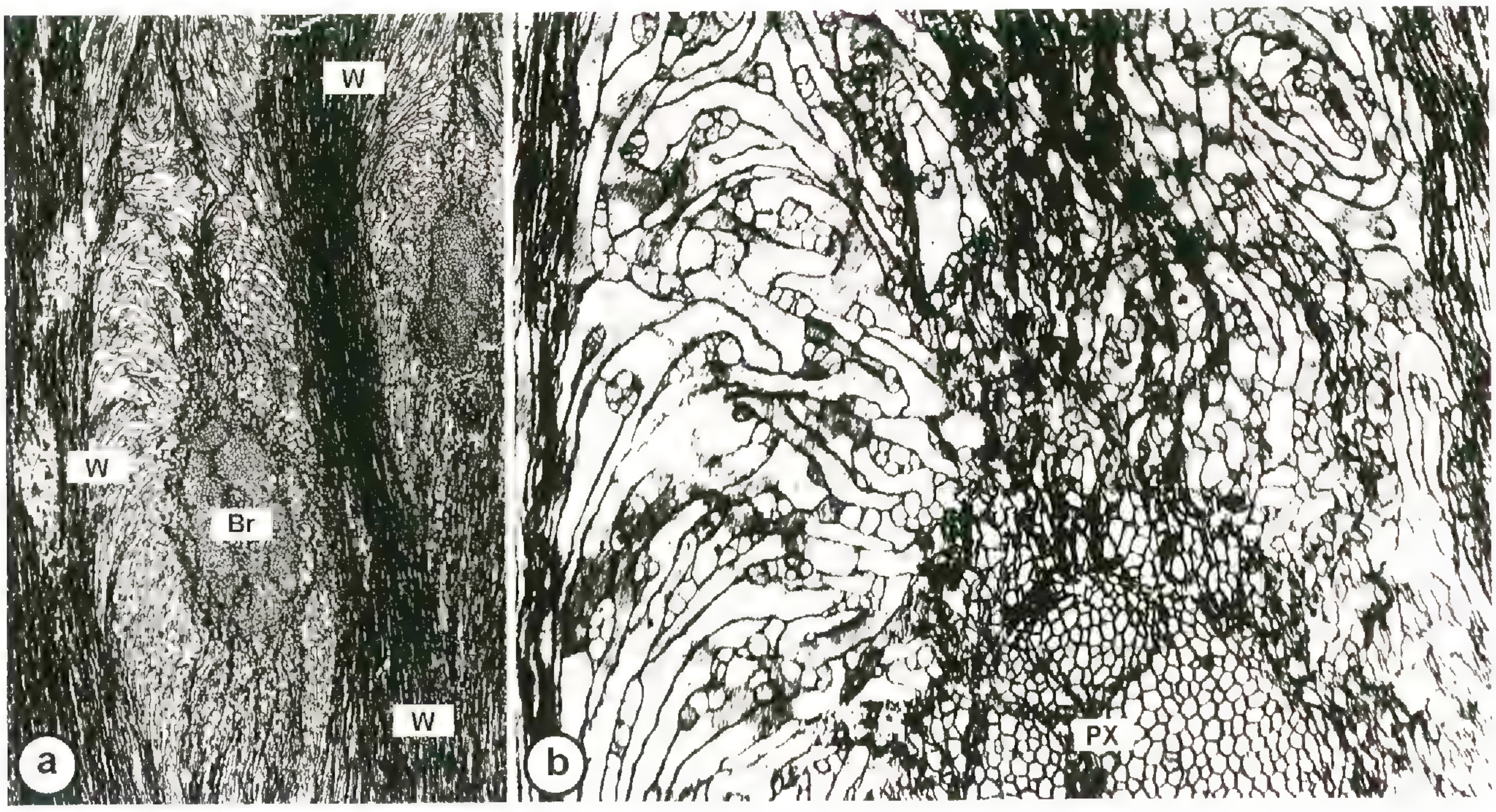

Figure 2. Secondary xylem of a *Callixylon whiteanum* Arnold stem in tangential section at the level of branch divergence. —a. Branch stele (Br) in cross section surrounded by wood (W) of stem, showing circular patterns of tracheids caused by disruption of polar auxin transport and pooling of auxin above branch. —b. Enlargement of wood distal to randomly arranged tracheids of the primary xylem (PX) of the diverging branch stele, showing distorted tracheids and ray cells forming circular patterns.

study of development in fossil plants, Eggert (1961) documented a complex mode of primary and secondary growth for the vascular tissues of lepidodendralean lycophytes (Fig. 3A). Eggert determined that the shoot system of lepidodendralean trees had a primary vascular system that increased in size up to the level of the first branch and then decreased distally (Fig. 3B). By contrast, the thickness of the secondary xylem (i.e., wood; zone with horizontal lines in Fig. 3B) decreases distally (i.e., from the base of the stem), with the apical regions of the branches having no secondary vascular tissues (Fig. 3B). This developmental pattern demonstrates that the wood is the product of secondary growth similar to that of living seed plants in some respects but different in others (Cichan, 1985). Therefore, primary growth and secondary growth are products of independent meristems in arborescent rhizomorphic lycophytes (Eggert, 1961; Cichan, 1985). Up to the present, however, there has been no definitive evidence for the structure or developmental regulation of a lepidodendralean-type vascular cambium.

Paralycopodites brevifolius *(Williamson) DiMichele.* *Paralycopodites* Morey & Morey is a stratigraphically long-ranging genus of arborescent rhizomorphic lepidodendralean lycophytes that is roughly similar to the plant reconstructed in Figure 3A. *Paralycopodites* has a long trunk and much-branched crown, with bisporangiate cones that terminate lateral branches and a highly branched stigmarian rooting system (DiMichele, 1980: but with infrequent dichotomous branching and numerous deciduous lateral branches in the crown as compared to the plant in Fig. 3A). Branches of *Paralycopodites* that have steles in the range of 2–3 cm in diameter (Fig. 4A) display a large pith surrounded by a medulated protostele of primary tracheids with a smooth outer margin, and abundant secondary xylem that comprises a relatively dense wood composed of tracheids and infrequent narrow rays (Fig. 4B). At the level where a branch base diverges from the stem stele, the xylem of the branch consists of a C-shaped stele of tracheids and adaxially oriented parenchyma (Fig. 4B) that becomes a radial stele at more distal levels (DiMichele, 1980). Tangential sections of *Paralycopodites* stems near the periphery of the wood (i.e., along line c–c of Fig. 4A) show regularly arranged, long, straight tracheids and interspersed narrow rays (Fig. 4B, at left and bottom). Immediately distal to the branch trace the axial files of tracheids are disrupted, many tracheids are distorted, and others extend obliquely or horizontally (Fig. 4B, at upper right). By contrast, tracheids immediately below the branch form regular axial files that are similar in pattern to those in non-branching regions (Fig. 4B, at bottom right).

Chaloneria cormosa *Pigg & Rothwell.* Compared to *Paralycopodites* and other lepidodendralean trees, *Chaloneria cormosa* is a relatively small and unbranched rhizomorphic lycophyte of Upper Pennsylvanian age that grew to about 2 m tall (Fig. 3C).

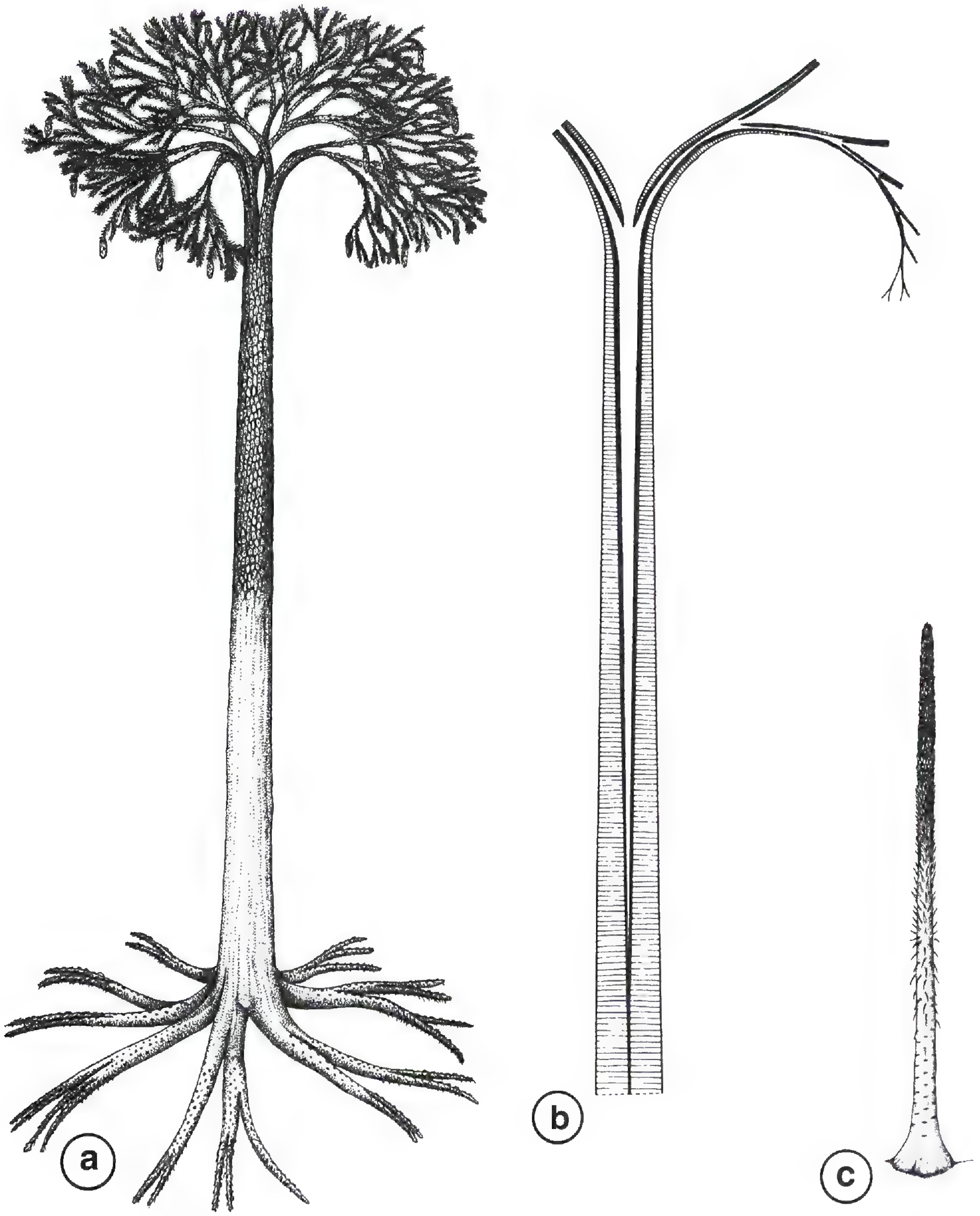

Figure 3. Diagrams of fossilized woody lepidodendralean and isoetalean lycophytes from the Pennsylvanian of North America and Europe. —a. Lepidodendralean tree ca. 8–10 m tall, showing elongated trunk and branching crown with terminal cones and branched rhizomorphic rooting system assignable to the morphogenus *Stigmaria* Goeppert. Modified from Stewart & Rothwell, 1993. —b. Diagrammatic representation of growth architecture of lepidodendralean trees. Note that the size of the primary xylem (black = primary xylem; white = pith) increases up to first branch and then decreases distally with each subsequent branch. Also note that the thickness of secondary xylem (horizontal lines) decreases from base of stem distally. Modified from Eggert, 1961. Used with permission from Cambridge University Press. —c. Unbranched plant of the isoetalean *Chaloneria cormosa* Pigg & Rothwell ca. 2 m tall, showing cormose base, vegetative basal region of stem, and fertile apex. Modified from Pigg and Rothwell, 1981.

This species produced vegetative leaves toward the base, displayed alternating zones of microsporangiate and megasporangiate sporophylls distally, and had a corm-like base similar to the living quillwort, *Isoetes* (Fig. 3C; Pigg & Rothwell, 1981). Secondary xylem occurs at and near the base of the *Chaloneria* Pigg & Rothwell plant (Fig. 4C), but the secondary xylem diminishes in thickness rapidly and is absent from all but the most basal regions of the stem (Pigg & Rothwell, 1981).

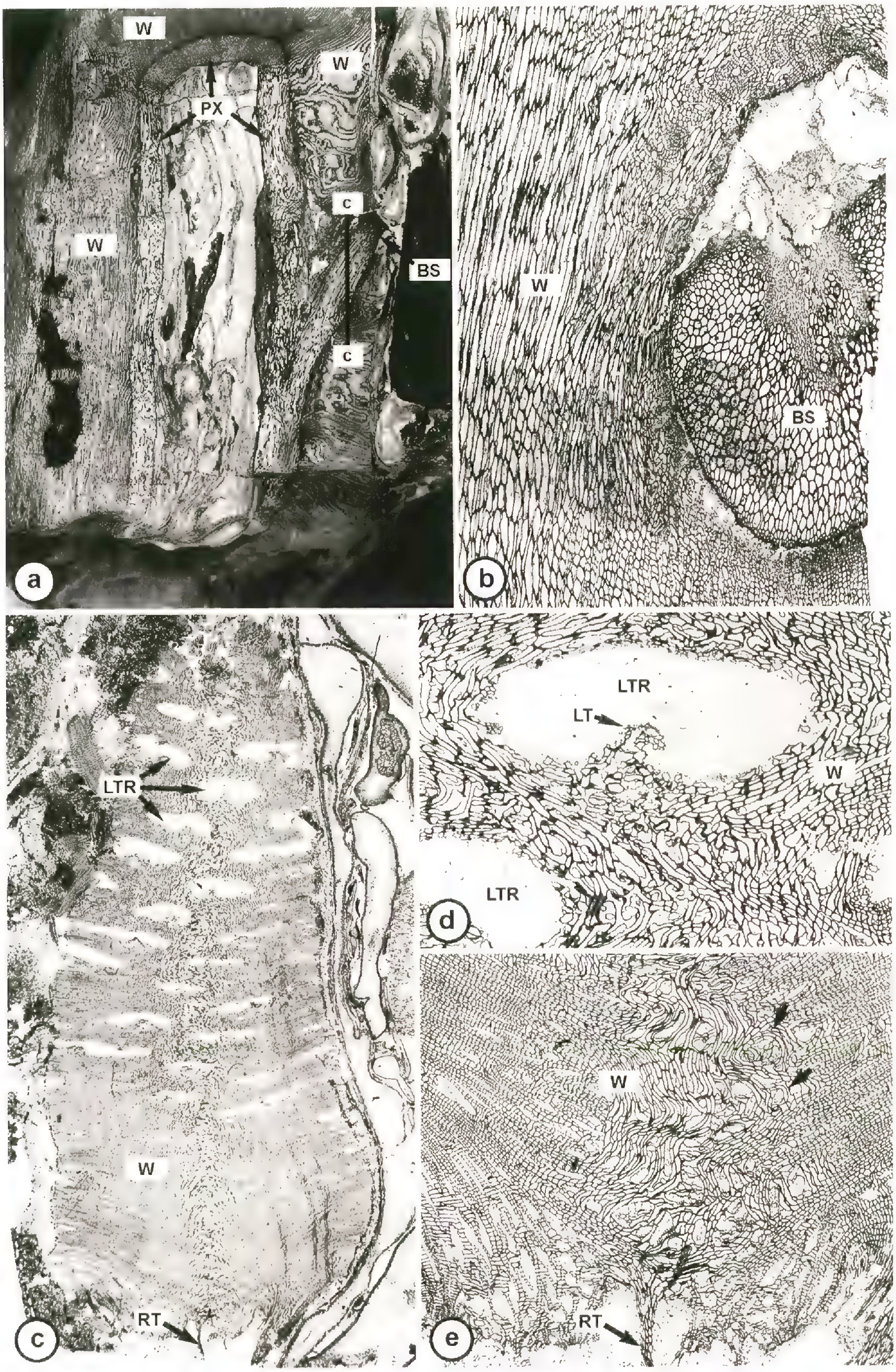

Figure 4. Anatomical sections through stems of fossilized woody rhizomorphic lycophytes showing evidence for axial polar auxin flow in regulation of secondary vascular tissue production. —a. *Paralycopodites brevifolius* (Williamson) DiMichele stem from the lower Middle Pennsylvanian of eastern Kentucky, showing radial view through level of a branching at coal ball surface. The pith region is surrounded by a cylinder of primary xylem (PX; also seen in cross section at the top and bottom), which is enclosed in a cylinder of wood (W). Note diverging branch stele (BS) at right. Line c–c indicates plane of section shown in part c. —b. Tangential section through secondary xylem of stem, cut through line c–c of part a. Note that the

Tangential sections near the base of a *Chaloneria* stem show secondary xylem that is perforated by helically arranged leaf traces (Fig. 4C), each within a broad ray (Fig. 4D). At the base of the plant (Fig. 4C), root traces diverge from the rounded bottom of the woody zone (Fig. 4E, at "RT"). An absence of leaf traces at the base of the plant indicates that leaves were not produced in that region (Fig. 4C, at bottom; 4E). Tangential sections through the secondary xylem of *Chaloneria* reveal radial files of tracheids that follow a sinuous course around the large leaf-trace rays (Fig. 4C, D). In some areas, particularly immediately distal to each ray, the tracheids are highly distorted, with some tracheids being branched and others forming circular patterns (Fig. 4D). Highly distorted patterns of tracheids are also present at the base of the plant, where branched tracheids and swirls of tracheids are common (Fig. 4E, at arrows).

ARBORESCENT WOODY EQUISETOPHYTES

Arborescent equisetophytes of the Pennsylvanian coal swamps were essentially giant woody *Equisetum* L.-like plants (Fig. 5A; Good, 1975) that produced wood that is assignable to three morphogenera including *Arthropitys* (Taylor & Taylor, 1993). In cross sections, *Arthropitys* stems have a large pith surrounded by a ring of primary xylem bundles (Fig. 5B). Conspicuous carinal canals characterize the primary xylem and vallecular canals occur in the cortex (Figs. 5B, 6A), both of which are virtually identical to comparable structures in living species of *Equisetum* (Fig. 6D). By comparison to *Equisetum*, *Arthropitys* stems range much larger in diameter. *Arthropitys* stems also differ from *Equisetum* by the production of abundant secondary xylem that forms wedges of radially aligned tracheids separated by wide parenchymatous rays (Figs. 5B, 6A; Stewart & Rothwell, 1993).

At the level of a node, tangential sections through the secondary xylem of *Arthropitys* stems show branch bases that are positioned slightly above and on alternating radii with leaf traces (Fig. 6B). The branch base shows a large hollow pith that is surrounded by a ring of carinal canals and wood, all of which are similar to those in the stem from which the branch has arisen (cf. Fig. 6A, B). As seen in tangential sections, the secondary xylem of the stem typically consists of regular axial files of long and straight tracheids and interspersed narrow rays (Fig. 6B, at sides) except where leaf traces and especially branch junctions are positioned. Tracheids of the secondary xylem extend around leaf traces (Fig. 6B, at bottom arrows), often showing a small area of tracheids that form a circular pattern (Fig. 6C, at bottom left).

Evidence of regulation of vascular differentiation by the axial polar auxin flow is more pronounced where the wood extends around the much larger branch bases (Fig. 6B). Cells distal to and extending around to the sides of the branch stele form distinct swirls and circular patterns (Fig. 6C), and there are much larger numbers of ray cells than in other areas of the secondary xylem. Also, some tracheids branch (Fig. 6B, arrow at top).

SYSTEMATIC SCOPE OF POLAR AUXIN REGULATION IN WOOD DEVELOPMENT

Fossil wood assigned to the genus *Callixylon* (Fig. 2A, B) is produced by the plant *Archaeopteris* (Beck, 1960), which is a member of the Progymnospermopsida, a paraphyletic grade subtending seed plants in the clade that is known as lignophytes (Fig. 1; Crane, 1985; Rothwell & Serbet, 1994). The discovery of a common regulatory pathway for the development of secondary vascular tissues in progymnosperms and seed plants strengthens the hypothesis that such tissues are homologous and provides additional support for the definition of the lignophyte clade (Fig. 1). Although large woody trees with substantial secondary vascular tissue production are restricted to seed plants in the modern flora, the fossil record includes highly branched, woody equisetophyte and lycophyte trees that were prominent components of wetland forests during the Paleozoic Era (Taylor & Taylor, 1993).

Data developed in the current study reveal cellular patterns in the secondary xylem of both Paleozoic lycophytes (*Paralycopodites* and *Chaloneria*) and equisetophytes (*Arthropitys*) that are diagnostic for regulation of cambial growth and secondary xylem production by polar axial auxin flow among living

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tracheids distal to the branch stele (BS) are distorted, whereas those below the branch stele are not. —c. Tangential section at base of *Chaloneria cormosa* Pigg & Rothwell from the Upper Pennsylvanian of Ohio, showing helically arranged leaf traces with large leaf-trace rays (LTR) distal to a cormose plant base. Note that no leaf traces or rays traverse the wood (W) at the base of the plant. —d. Enlargement of wood in stem region of part c (W), showing diverging leaf trace (LT) surrounded by a wide LTR. Note contorted and circular patterns of tracheids distal to the LTR. —e. Enlargement of part c at base of plant showing distorted and circular patterns of tracheids (at arrows) in the wood (W) above a diverging rootlet trace (RT) at the base of the plant.

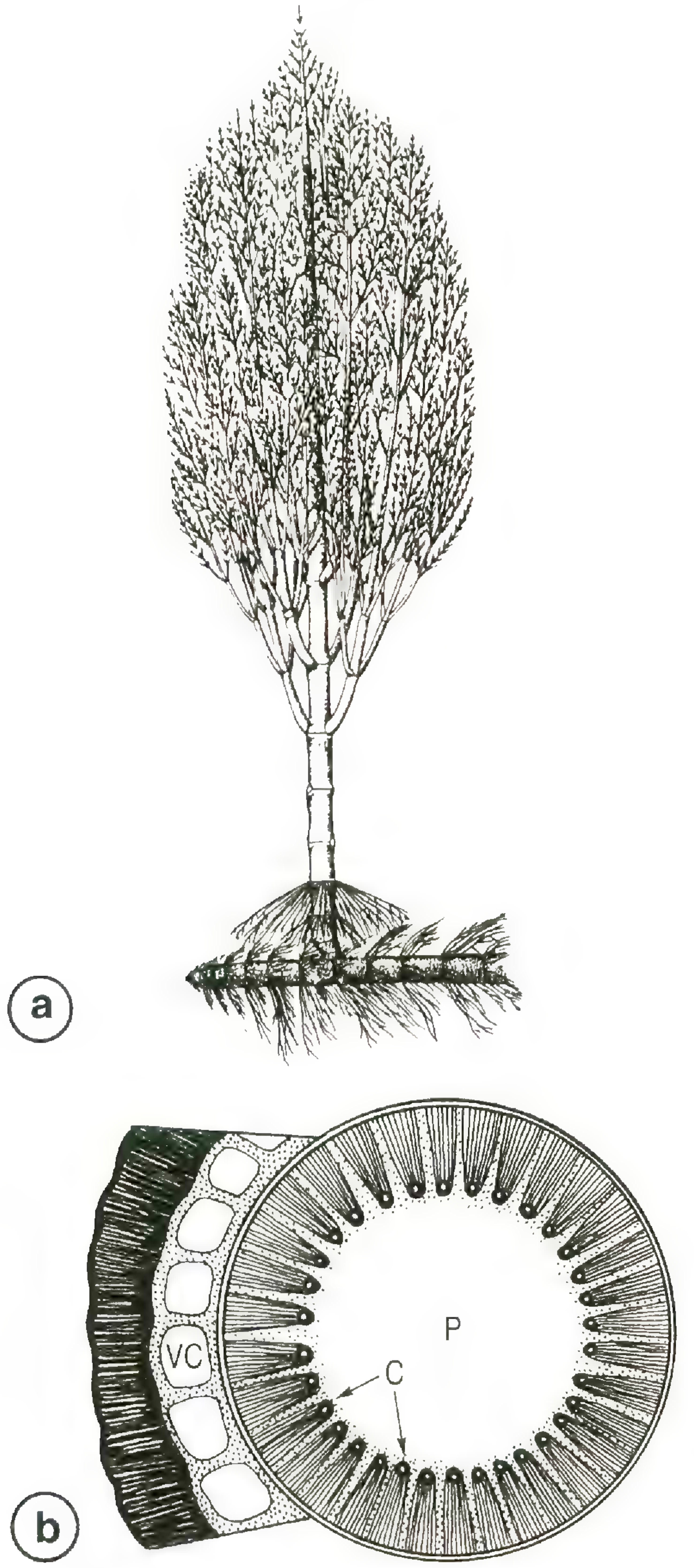

Figure 5. Diagrams of fossilized calamitalean equisetophytes from the Pennsylvanian of North America and Europe. —a. Calamitalean tree ca. 6–8 m tall, diverging from underground rhizome. Modified from Stewart and Rothwell, 1993. —b. Cross section of *Arthropitys* stem showing characteristic *Equisetum*-like anatomy and secondary xylem. Important features include hollow pith (P), carinal canals (C) surrounded by wood, and vallecular canals (VC) in cortex. Modified from Stewart and Rothwell, 1993.

seed plants. Distorted files of axial secondary xylem cells, branched tracheids, and circular patterns of tracheids are associated with leaf trace divergence in *Chaloneria* (Fig. 4C, D), reflecting swirling polar auxin currents above disruptions in the vascular cambium that are every bit as pronounced as those that result from obstructions to auxin flow in the cambium of living and fossil lignophytes (Fig. 2). These patterns document that axial polar auxin flow was involved in the regulation of wood development in lycophytes as well as in lignophytes (Fig. 1; Rothwell & Lev-Yadun, 2005). Likewise, the occurrence of well-developed diagnostic cellular patterns in the secondary xylem of *Arthropitys* demonstrates the regulation of secondary xylem development by the axial polar auxin flow in extinct equisetophytes. Together, these data from lignophyte, equisetophyte, and lycophyte taxa reveal that polar auxin transport facilitates a common mode of developmental regulation for secondary vascular tissues across the vascular plant evolutionary tree (Fig. 1). That is to say, polar auxin regulation of wood development is common to both major clades of living vascular plants, Lycophytina and Euphyllophytina (which includes both equisetophytes and lignophytes; Fig. 1).

VARIATIONS IN FOSSIL EVIDENCE FOR REGULATION BY AXIAL POLAR AUXIN FLOW

Although anatomical evidence for the regulation of secondary vascular tissue production by axial polar auxin flow is present in all three major clades of vascular plants that have produced abundant wood through time (i.e., lignophytes, equisetophytes, lycophytes; Fig. 1), there are significant variations in the degree of development and in the locations of evidence among representatives of the various clades. In all of the examples studied to date, evidence for regulation of vascular differentiation by deviation from the typical orientation of the axial polar auxin flow is preserved in the secondary xylem above the positions where there would have been obstructions to auxin flow in the cambium during tissue patterning (Figs. 2, 4, 6). Lignophytes (Fig. 2B), such as the extinct progymnosperm *Archaeopteris* (represented here by the morphotaxon *Callixylon*, Fig. 2), living species of both conifers and dicotyledonous flowering plants (Rothwell & Lev-Yadun, 2005: figs. 2, 3), and fossil equisetophytes such as *Arthropitys* (Fig. 6B) all show obvious regions of distorted tracheids, branched tracheids, and circular patterns of cells distal to branch junctions. The equisetophytes *Arthropitys* and *Archaeocalamites* Stur also show evidence of circular patterns associated with leaf trace divergence (Fig. 6B; Smoot et al., 1982), demonstrating that small obstructions in polar auxin regulation were sufficient to produce the diagnostic fingerprint in this clade by the Mississippian Period (ca. 330 Ma).

Files of axial tracheids of the secondary xylem distal to branches in *Paralycopodites* are also disrupted above the branches, but the extent of the zone is much smaller and the distortions are far less pronounced (Fig. 4B, at top) than in the other plant clades studied. This subtle evidence for regulation of secondary xylem differentiation by the axial polar auxin flow in *Paralycopodites* is most easily recognized by noting differences in the wood immediately above and below a branch. By comparison to the subtly distorted patterns distal to a branch, files of cells below the same branch (cf. cells above and below the branch in Fig. 4B) of *Paralycopodites* show no evidence of distortion at all. Were it not for the obvious disruptions of cell files with branched tracheids and circular patterns of cells present in the wood of other lycophytes (e.g., *Chaloneria*; Fig. 4D, E), it would be difficult to interpret regulation of secondary xylem development by the axial polar auxin flow in fossil isoetalean lycophytes with certainty.

Chaloneria cormosa is unbranched and smaller than lepidodendraleans (cf. Fig. 3). *Chaloneria* also has a cormose base like living *Isoetes*, rather than the elongated and much-branched stigmarian rooting system that characterizes *Paralycopodites* and other members of the Lepidodendrales (Fig. 3A). Nevertheless, evidence for the regulation of secondary vascular tissue development by the axial polar auxin flow is well represented in *Chaloneria* (Fig. 4C–E). Disrupted cellular patterns, branched tracheids, and swirling patterns of tracheids occur in association with the wide rays that accompany diverging leaf traces (Fig. 4C, D). Because of the extremely short internodes and numerous helically arranged leaves of *Chaloneria* (Fig. 4C), the closely spaced wide leaf-trace rays must have produced a considerable obstruction to the axial polar auxin flow in the developing vascular cambium. That complex pattern of obstruction probably accounts for the occurrence of contorted and branched tracheids around several sides of many rays (Fig. 4D).

One of the most distinctive and initially unexpected occurrences of an axial polar auxin flow fingerprint in fossils is found at the base of the *Chaloneria* plant, where particularly well-developed circular patterns are present (Fig. 4C, E). Such patterns were first recognized as being distinctive and bilateral in distribution before the role of the axial polar auxin flow in regulation of secondary xylem development was understood (Pigg & Rothwell, 1979). Because those circular patterns are present in a region that is below all typical obstructions in polar auxin flow that would have been caused by the divergence of branch junctions and leaf traces, their occurrence must be attributable to another phenomenon. We know from wounding and partial girdling experiments in oak (*Quercus ithaburensis* Decne. and *Q. calliprinos* Webb) that circular vascular tissues are also formed above any large obstacle to the polar axial auxin flow in tree trunks (Lev-Yadun & Aloni, 1991). An understanding of the unique morphology of rhizomorphic lycophytes provides a plausible explanation for the formation of the circular tissues at the base of *Chaloneria* plants.

Although some questions about the homologies of the rooting system of *Isoetes* remain among authors who work primarily with living species (Yi & Kato, 2001), there now is overwhelming evidence that the bipolar growth and rooting systems of isoetalean lycophytes are unique among all vascular plants. Evidence from the developmental morphology of *Isoetes* (Karrfalt & Eggert, 1984) and from the structure, development, and embryology of fossil species (Frankenburg & Eggert, 1969; Phillips, 1979; Karrfalt, 1980) supports the hypothesis that isoetalean lycophytes have no true roots that could be homologous to the roots of *Selaginella* P. Beauv., other non-rhizomorphic lycophytes, or any other group of vascular plants (Rothwell & Erwin, 1985). Rather, the rhizomorph of *Isoetes* and all of the isoetalean lycophytes is homologous to a shoot system that has become highly modified for rooting the plant. This means that the structures commonly referred to as the roots of *Isoetes* are actually leaves that have been modified for rooting (Frankenburg & Eggert, 1969).

That interpretation is both explained by and further strengthened by the distinct evidence for disruption in polar auxin flow at the very base of the *Chaloneria* plant. If, indeed, *Chaloneria* and other rhizomorphic lycophytes have no true roots, as interpreted from the fossil record (Frankenberg & Eggert, 1969; Rothwell & Erwin, 1985), then basally flowing auxin would have nowhere to go after reaching the cormose base of the plant. The auxin must have pooled and swirled in the cambial zone at the base of the trunk, thus inducing the distinctive anatomical patterns in the wood at the base of *Chaloneria* specimens (Fig. 4C, E; Pigg & Rothwell, 1979). Anatomical descriptions of the basal regions of other fossil rhizomorphic lycophytes with cormose bases are scarce. However, in a genus that is even more ancient than *Chaloneria* (i.e., the 360-million-year-old Lower Carboniferous lycopohyte *Trabicaulis flabellilignis* Meyer-Berthaud; Meyer-Berthaud, 1984), there is evidence for disruptions of tracheid patterning similar to those of *Chaloneria* both above leaf trace divergences (i.e., Meyer-Berthaud, 1984: fig. 8) and at the base of the plant (i.e., Meyer-Berthaud, 1984: Planch 3, fig. 4). These data reveal that regulation of secondary vascular tissue production by the axial polar auxin flow was probably widespread among rhizomorphic lycophytes with cormose bases. Moreover, there is now additional compelling evidence that the most distinctive synapomorphy for isoetalean lycophytes, the rhizomorph, represents a shoot system that has been modified for rooting.

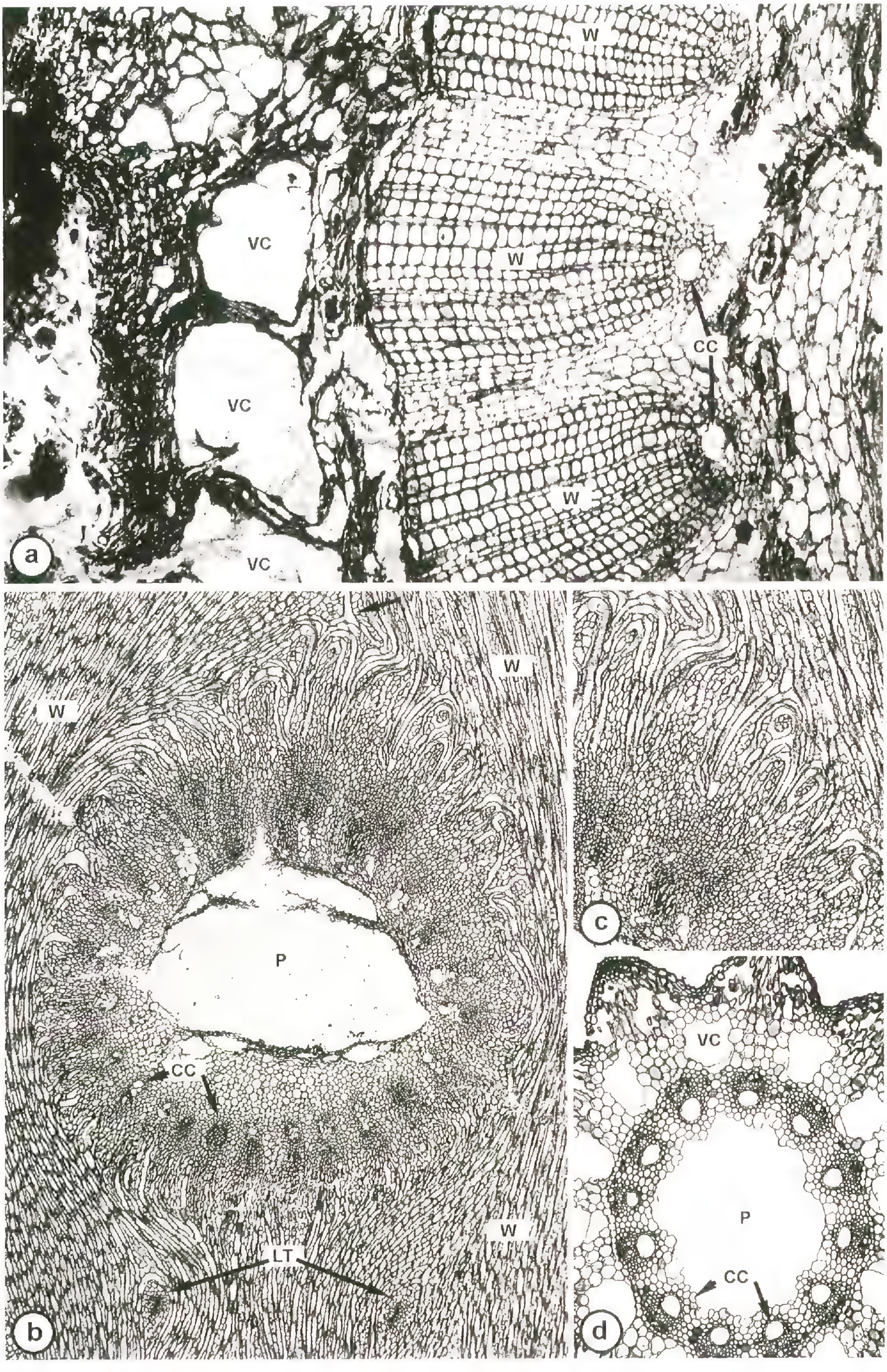

Figure 6. Anatomical sections through stems of large, woody equisetophytes assignable to the Paleozoic Calamitales (a–c) and living *Equisetum* L. (d). —a. Cross section through segment of *Arthropitys* Goeppert stem from Westphalian A sediments of England, with pith at right and cortex at left. Carinal canals (CC) in the primary xylem and vallecular canals (VC) in the cortex that are comparable to those of living *Equisetum* (d). Note the well-developed segments of secondary xylem that are separated by wide pith rays. This wood (W) shows both long radial files of secondary tracheids and interspersed narrow parenchymatous rays. —b. Tangential section through wood of *Arthropitys* at the level of a node. Diverging branch base in cross section shows a

Fossil Evidence for Growth Regulation in the Evolution of Plants

Methodology for recognizing specific developmental regulatory pathways from diagnostic structural features of fossil plants has recently begun to emerge as an additional approach for improving the understanding of evolution through geological time and for testing hypotheses of evolution and phylogenetic relationships among major clades of vascular plants (Rothwell & Lev-Yadun, 2005). This represents a new approach, the first application of which is based on our knowledge that there are disrupted files of contorted and branched cells that form circular patterns immediately distal to branches and large leaf-trace rays in living seed plants. Because those patterns are known to represent an anatomical fingerprint for the axial polar auxin flow that regulates patterns of secondary vascular tissue development (Sachs & Cohen, 1982; Hejnowicz & Kurczyńska, 1987; Lev-Yadun & Aloni, 1990), the discovery of circular patterns in the wood of a 375-million-year-old species of *Archaeopteris* has provided the first direct evidence that progymnosperms and seed plants share a common mechanism of growth regulation for secondary vascular tissues. Those data also reveal that auxin-mediated secondary vascular tissue development is a synapomorphy for lignophytes (i.e., a clade that includes seed plants and their progymnospermous sister groups).

Implications for the Evolution of Wood Across Vascular Plants

These newly developed data for the fossil evidence for regulatory mechanisms of wood production now can be combined with an understanding of phylogenetic pattern and first occurrences of secondary vascular tissues in divergent clades to develop hypotheses for the evolution of secondary vascular tissues across the vascular plant evolutionary tree. Although the overall topology of vascular plant relationships remains a subject of controversy (Rothwell & Nixon, 2006), there is wide agreement about some of the deep internal nodes of the evolutionary tree. Living plants and their closest extinct sister groups comprise two major clades, Lycophytina and Euphyllophytina sensu Kenrick and Crane (1997), some representatives of which can be identified as early as the Late Silurian. This places the minimum age for divergence of lycophyte ancestors from euphyllophyte ancestors at about 410 million years, which is at least 35 million years earlier than the first occurrence of secondary vascular tissues in either clade (Fig. 1). Therefore, secondary vascular tissues in lycophytes and euphyllophytes must have evolved independently. Evidence for separate origins of secondary vascular tissues in equisetophytes and lignophytes is not as strong because of the current uncertainty about relationships among euphyllophytes (Fig. 1; Rothwell & Nixon, 2006). However, the first evidence for secondary vascular tissues in either equisetophytes or lignophytes is on the order of five million years younger than the minimum age of divergence between these two clades (Fig. 1). Therefore, it is also likely that secondary vascular tissues evolved independently in the ancestors of *Equisetum* and seed plants.

We now recognize that the developmental mechanism (i.e., involving polar axial auxin flow) for secondary vascular tissue production is common to lignophytes, equisetophytes, and lycophytes, but that the secondary vascular tissues evolved separately either in lycophytes and euphyllophytes or in all three clades (Fig. 1). Therefore, wood production in the major clades of vascular plants is the product of convergent evolution. The question remains whether the underlying regulatory mechanism (i.e., by the axial polar auxin flow) for secondary vascular tissues also evolved independently in the various clades or whether it was present in the genomes of all of the clades prior to the evolution of secondary vascular tissues.

Among living plants, this question has been addressed in a molecular survey of embryophytes and their algal sister groups (Cooke et al., 2002). Although conjugation rates and the complexity of auxin metabolism both increase toward the distal branches of the tree, these authors record that indoleacetic acid (IAA) metabolism is present in the bryophytic sister groups of vascular plants as well as all of the lycophytes and euphyllophytes that have been surveyed. Within the tracheophytes, auxin

←

hollow pith (P) surrounded by pith parenchyma and a ring of carinal canals (arrows at CC). Branch is situated just distal to, and on a radius that alternates with, radii upon which leaf traces (arrows at LT) are produced. Note that circular patterns of tracheids (indicative of the role of the axial polar auxin flow in regulation of secondary vascular tissue production) are positioned distal to the branch, but are not produced below the branch. Arrow at top of photo identifies branching tracheid. —c. Enlargement of disrupted wood pattern distal to branch in part b, showing swirls and circular patterns of cells. —d. *Equisetum arvense* L. stem in cross section, showing anatomical features (CC = carinal canal, P = pith, VC = vallecular canal) that are virtually identical to the primary tissues of *Arthropitys* (parts a and b).

metabolism is widely associated with primary vascular tissue production (Leyser & Berleth, 1999); it also may be associated with development of conducting tissues in bryophytes (Cooke et al., 2002).

The basal/apical ratio of auxin flow is one to two orders of magnitude lower in the only lycophyte studied, *Selaginella*, than it is in the euphyllophytes studied (Cooke et al., 2002: table 4). If that difference is characteristic of lycophytes as a whole, it would account for the much less obvious anatomical fingerprint for the role of axial polar auxin flow in the regulation of secondary xylem formation above the branches of *Paralycopodites* than in similar positions of the fossil euphyllophytes studied (i.e., *Archaeopteris* and *Arthropitys*). This ratio has yet to be determined for *Equisetum*. However, if the distinctive fingerprint present in the wood of *Arthropitys* (Fig. 6B) is correlated with levels of polar auxin flow, we hypothesize that future studies will document polar auxin metabolism in equisetophytes that is comparable to other euphyllophytes.

CONCLUSIONS

Until the recent recognition of structural fingerprints for axial polar auxin flow in fossil plants (Rothwell & Lev-Yadun, 2005), it has not been possible to employ data from the paleontological record to infer plant evolution from changes in developmental regulation. However, the discovery of developmentally diagnostic circular patterns in fossil secondary xylem reveals that secondary vascular tissue development was probably regulated by the axial polar auxin flow in all of the major clades of vascular plants that have produced woody trees through time. Because such patterns result from obstructions of the axial polar auxin flow in the vascular cambium, both the positions of the circular patterns and the extent of their development provide meaningful information about specifics of the auxin metabolism, the growth and development that has resulted from regulation by auxin, and the organography of the extinct plants. These data imply that the much lower basal/apical IAA transport ratios found in living *Selaginella* also characterized Paleozoic fossil lepidodendraleans and that the patterns preserved above branch junctions of Paleozoic fossil Calamitales are predictors of the regulation by the axial polar auxin flow in living *Equisetum* that is similar to other modern euphyllophytes. Likewise, the extensive development of circular patterns at the base of *Chaloneria* and similar Paleozoic lycophytes with a plant base similar to living *Isoetes* strongly supports the hypothesis that rhizomorphic lycophytes are rooted by a highly modified shoot system and that they lack true roots that are homologous to those of other vascular plants.

Although the fossil record now provides strong anatomical evidence that a common mode of developmental regulation underlies secondary vascular tissue production in all of the major clades of vascular plants that have produced extensive wood through time, the currently recognized pattern of phylogeny for tracheophytes reveals that secondary vascular tissues probably evolved independently in lycophytes, equisetophytes, and lignophytes. The occurrence of regulation by polar auxin flow of primary growth across the entire spectrum of vascular plants (Sachs, 1991; Scarpella et al., 2006) indicates that genes for this developmental regulatory mechanism were present in the herbaceous ancestors of all woody plants, although they were not expressed in circular patterns before the emergence of secondary vascular tissues (Rothwell & Lev-Yadun, 2005) and were available for exaptation to the new role of secondary vascular tissue development. Therefore, while the secondary xylem of lignophytes, equisetophytes, and lycophytes is not homologous (i.e., inherited from a common ancestor that produced secondary xylem), the genes that underlie the regulation of wood production by the axial polar auxin flow in the three clades probably are homologous (i.e., they probably are paralogues).

As a result of the extensive extinction that has occurred within all ancient clades of vascular plants (Mishler, 2000), including the large woody trees of lycophytes and equisetophytes investigated in this study, there is a rapidly growing recognition that the in-depth understanding of evolution at both the genetic/regulatory and organismal levels relies heavily on paleontological data. Information presented in this study reveals that such data are, indeed, available from fossil plants. Although the work presented here is restricted to increasing our understanding of growth of secondary vascular tissues in terms of regulatory mechanism evolution, the rapidly growing number of newly developed techniques for recognizing developmental regulatory mechanisms from fossils (Boyce & Knoll, 2002; Rothwell et al., 2006; Sanders et al., 2007) promises to increase dramatically the breadth of such studies. In so doing, paleontology is becoming more intimately incorporated into the mainstream of plant evolutionary studies.

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